

What is claimed is:

1. A peptide comprising an amino acid sequence having a cleavage site specific for an enzyme having a proteolytic activity of prostate specific antigen, wherein the peptide is 20 or fewer amino acids in length.

2. The peptide of claim 1, wherein the sequence comprises: the amino acids

$X_5X_4X_3X_2X_1$ ,

wherein  $X_5$  is from 0 to 16 amino acids;  $X_4$  is serine, isoleucine, or lysine;  $X_3$  is serine or lysine;  $X_2$  is leucine, tyrosine or lysine; and  $X_1$  is glutamine, asparagine or tyrosine.

3. The peptide of claim 2, further comprising  $X_{-1}$  linked to  $X_1$ , wherein  $X_{-1}$  is from 1 to 10 amino acids.

4. The peptide of claim 2, wherein  $X_1$  is glutamine.

5. The peptide of claim 2, further comprising amino acid  $X_6$  linked to the amino terminus of  $X_5$ , wherein  $X_6$  is from 0 to 15 amino acids and wherein  $X_5$  is serine or lysine.

6. The peptide of claim 5, further comprising amino acid  $X_7$  linked to the amino terminus of  $X_6$ , wherein  $X_7$  is from 0 to 14 amino acids and wherein  $X_6$  is histidine or asparagine.

7. The peptide of claim 2, wherein  $X_{-1}$  comprises leucine.

8. The peptide of claim 6, wherein the amino acid sequence is selected from the group consisting of His-Ser-Ser-Lys-Leu-Gln, Glu-His-Ser-Ser-Lys-Leu-Gln, Gln-Asn-Lys-Ile-Ser-Tyr-Gln, and Glu-Asn-Lys-Ile-Ser-Tyr-Gln.

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1 ~~9~~ The peptide of claim 1, further comprising a capping group attached to the N-terminus  
2 of the peptide, the group inhibiting endopeptidase activity on the peptide.

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10. The peptide of claim 9, wherein the capping group is selected from the group consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl and succinyl substituents.

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1 ~~11.~~ The peptide of claim 1, wherein the cleavage of the peptide by the enzyme yields at  
2 least 5 picomoles of cleaved peptide per minute per 200 picomoles of enzyme.

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12. The peptide of claim 1, wherein the cleavage of the peptide in human serum yields at most 2.0 picomoles of cleaved peptide per minute.

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13. A peptide of claim 1, further comprising an added substituent which renders the peptide water-soluble.

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14. A peptide of claim 13, wherein the added substituent is a polysaccharide.

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15. A peptide of claim 14, wherein the polysaccharide is selected from the group consisting of modified or unmodified dextran, cyclodextrin, and starch.

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1 ~~16.~~ A peptide of claim ~~2~~, further comprising an antibody attached to the amino terminus  
2 of X<sub>5</sub>, or X<sub>4</sub> when X<sub>5</sub> is 0.

1 17. A peptide composition comprising a plurality of peptides, each peptide comprising an  
2 amino acid sequence having a cleavage site specific for an enzyme having a proteolytic  
3 activity of prostate specific antigen, wherein each peptide has 20 or fewer amino acids.

18. A polynucleotide encoding the peptide of claim 1.

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1 19. A composition comprising a prodrug, the prodrug comprising  
2 a therapeutically active drug; and  
3 a peptide of claim 1,  
4 wherein the peptide is linked to the therapeutically active drug to inhibit the  
5 therapeutic activity of the drug, and wherein the therapeutically active drug is cleaved  
6 from the peptide upon proteolysis by an enzyme having a proteolytic activity of prostate  
7 specific antigen (PSA).

1 20. The composition of claim 19, wherein the peptide is linked directly to the therapeutic  
2 drug.

1 21. The composition of claim 20, wherein the peptide is linked directly to a primary  
amine group on the drug.

22. The composition of claim 19, wherein the peptide is linked to the therapeutic drug via  
a linker.

23. The composition of claim 22, wherein the linker is an amino acid sequence.

24. The composition of claim 23, wherein the linker comprises a leucine residue.

1 25. The composition of claim 19, wherein the therapeutically active drug inhibits a  
2 SERCA pump.

1 26. The composition of claim 25, wherein the therapeutically active drug is selected from  
2 the group of primary amine containing thapsigargin or thapsigargin derivatives.

1 27. The composition of claim 19, wherein the therapeutically active drug intercalates into  
2 a polynucleotide.

28. The composition of claim 27, wherein the therapeutically active drug is an anthracycline antibiotic.

29. The composition of claim 28, wherein the therapeutically active drug is selected from the group consisting of doxorubicin, daunorubicin, epirubicin and idarubicin.

30. The composition of claim 19, wherein the peptide is His-Ser-Ser-Lys-Leu-Gln-Leu.

31. The composition of claim 19, wherein the therapeutic drug is a compound belonging to the group of thapsigargins which have been derivatized with a moiety containing a primary amine group, the peptide is His-Ser-Ser-Lys-Leu-Gln, and the linker is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted  $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{Ar}-\text{NH}_2$ ,  $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{Ar}-\text{NH}_2$ ,  $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$  and  $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$ , wherein  $n1$  and  $n2$  are from 0 to 5, Ar is any substituted or unsubstituted aryl group, and attachment of  $\text{NH}_2$  to Ar is in a ortho, meta or para position with respect to the remainder of the linker.

32. The composition of claim 19, wherein the therapeutically active drug has an  $\text{IC}_{50}$  toward ER  $\text{Ca}^{2+}$ -ATPase of at most 500 nM.

33. The composition of claim 32, wherein the therapeutically active drug has an  $\text{IC}_{50}$  toward ER  $\text{Ca}^{2+}$ -ATPase of at most 50 nM.

34. The composition of claim 19, wherein the therapeutically active drug has an  $\text{LC}_{50}$  toward PSA-producing tissue of at most 20  $\mu\text{M}$ .

35. The composition of claim 34, wherein the therapeutically active drug has an  $\text{LC}_{50}$  toward PSA-producing tissue of less than or equal to 2.0  $\mu\text{M}$ .

1 36. The composition of claim 19, wherein cleavage of the peptide by the enzyme yields  
2 at least 5 picomoles of cleaved peptide per minute per 200 picomoles of enzyme.

1 37. The composition of claim 19, wherein cleavage of the peptide in human serum yields  
2 at most 2.0 picomoles of cleaved peptide per minute.

1 38. The composition of claim 19, further comprising an added substituent which renders  
2 the composition water soluble.

1 39. The composition of claim 38, wherein the added substituent is a polysaccharide.

1 40. The composition of claim 39, wherein the polysaccharide is selected from the group  
consisting of modified or unmodified dextran, cyclodextrin and starch.

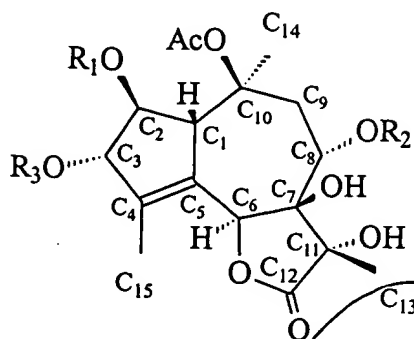
41. A therapeutically active sesquiterpene- $\gamma$ -lactone derivative containing a primary  
amine.

42. The derivative of claim 41, wherein the sesquiterpene- $\gamma$ -lactone is a thapsigargin  
derivative.

43. The thapsigargin derivative of claim 42, further comprising a boc protecting group.

1 44. The thapsigargin derivative of claim 42, wherein the derivative is linked to an  
2 antibody.

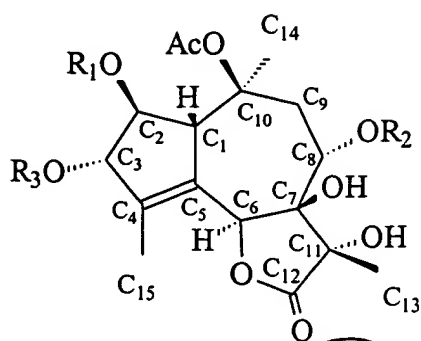
45. The thapsigargin derivative of claim 42, having the following structure



wherein  $R_1$  is a primary amine-containing alkanoyl, alkenoyl, or arenoyl substituent,  $R_2$  is an alkanoyl, alkenoyl, or arenoyl substituent, and  $R_3$  is an alkanoyl or alkenoyl substituent.

46. The thapsigargin derivative of claim 45, wherein  $R_1$  is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted  $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{Ar}-\text{NH}_2$ ,  $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{Ar}-\text{NH}_2$ ,  $\text{CO}-(\text{CH}_2)_{n2}-(\text{CH}=\text{CH})_{n1}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$  and  $\text{CO}-(\text{CH}=\text{CH})_{n1}-(\text{CH}_2)_{n2}-\text{CO}-\text{NH}-\text{Ar}-\text{NH}_2$ , wherein  $n1$  and  $n2$  are from 0 to 5, and Ar is any substituted or unsubstituted aryl group.

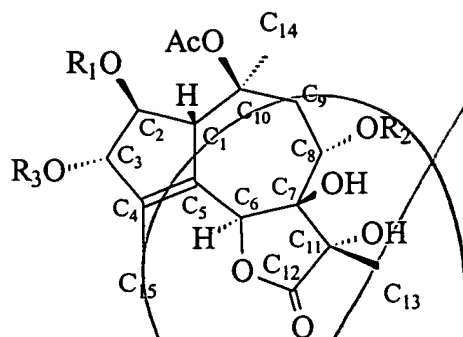
1 47. The thapsigargin derivative of claim 42, having the following structure



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wherein R<sub>1</sub> is an alkanoyl, alkenoyl, or arenoyl substituent, R<sub>2</sub> is a primary amine-containing alkanoyl, alkenoyl, or arenoyl substituent, and R<sub>3</sub> is an alkanoyl or alkenoyl substituent.

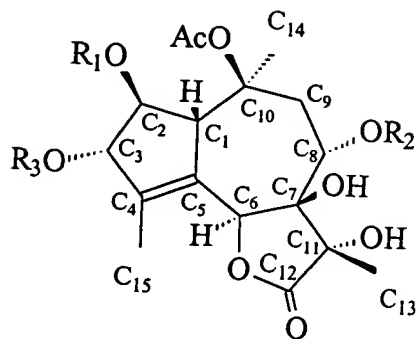
48. The thapsigargin derivative of claim 47, wherein R<sub>2</sub> is selected from the group consisting of unsubstituted or alkyl-, aryl-, halo-, alkoxy-, alkenyl-, amido- or amino-substituted CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>-(CH=CH)<sub>n1</sub>-Ar-NH<sub>2</sub>, CO-(CH<sub>2</sub>)<sub>n2</sub>-(CH=CH)<sub>n1</sub>-CO-NH-Ar-NH<sub>2</sub> and CO-(CH=CH)<sub>n1</sub>-(CH<sub>2</sub>)<sub>n2</sub>-CO-NH-Ar-NH<sub>2</sub>, wherein n<sub>1</sub> and n<sub>2</sub> are from 0 to 5, and Ar is any substituted or unsubstituted aryl group.

49. The thapsigargin derivative of claim 48, having the following structure



wherein  $R_2$  is  $\text{CO-CH=CH-Ph-p-NH}_2$ , wherein  $\text{Ph-p-NH}_2$  is the para-aminophenyl substituent.

1 50. The thapsigargin derivative of claim 48, having the following structure



wherein R<sub>2</sub> is CO-CH<sub>2</sub>-CH<sub>2</sub>-Ph-p-NH<sub>2</sub> wherein Ph-p-NH<sub>2</sub> is the para-aminophenyl substituent.

51. The thapsigargin derivative of claim 42, wherein the derivative has an IC<sub>50</sub> toward ER Ca<sup>2+</sup>-ATP-ase of at most 500 nM.

52. The thapsigargin derivative of claim 51, wherein the derivative has an IC<sub>50</sub> toward ER Ca<sup>2+</sup>-ATP-ase of at most 50 nM.

53. The thapsigargin derivative of claim 42, wherein the derivative has an LC<sub>50</sub> toward PSA-producing tissue of at most 20 μM.

54. The thapsigargin derivative of claim 53, wherein the derivative has an LC<sub>50</sub> toward PSA-producing tissue of at most 2.0 μM.

1 55. A method of producing a prodrug, the method comprising the step of linking  
2 a therapeutically active drug and  
3 a peptide of claim 1,  
4 wherein the linking of the peptide to the drug inhibits the therapeutic activity of  
5 the drug.

1 56. The method of claim 55, wherein the therapeutically active drug has a primary amine.

1 57. The method of claim 55, wherein the prodrug contains a linker between the peptide  
2 and the drug.

1 58. The method of claim 57, wherein the linker comprises Leu.

1 59. The method of claim 55, wherein the peptide further comprises a capping group  
attached to the N-terminus of the peptide, the group inhibiting endopeptidase activity on  
the peptide.

1 60. The method of claim 59, wherein the capping group is selected from the group  
consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl, and succinyl  
substituents.

1 61. A method of treating a PSA-producing cell proliferative disorder, the method  
2 comprising administering the composition of claim 19 in a therapeutically effective  
3 amount to a subject having the cell proliferative disorder.

1 62. The method of claim 61, wherein the disorder is benign.

1 63. The method of claim 61, wherein the disorder is malignant.

1 64. The method of claim 63, wherein the malignant disorder is prostate cancer.

65. The method of claim 63, wherein the malignant disorder is breast cancer.

66. A method of detecting prostate specific antigen-producing tissue, the method comprising:

contacting the tissue with a composition comprising  
a detectably labeled peptide of claim 1 for a period of time sufficient to  
allow cleavage of the peptide; and  
detecting the detectable label.

67. The method of claim 66, wherein the peptide further comprises a capping group attached to the N-terminus of the peptide, the group inhibiting endopeptidase activity.

68. The method of claim 67, wherein the capping group is selected from the group consisting of acetyl, morpholinocarbonyl, benzyloxycarbonyl, glutaryl, and succinyl substituents.

69. The method of claim 66, wherein the detectable label is a fluorescent label.

70. The method of claim 69, wherein the fluorescent label is selected from the group consisting of 7-amino-4-methyl coumarin, 7-amino-4-trifluoromethyl coumarin, rhodamine 110, and 6-aminoquinoline.

71. The method of claim 66, wherein the detectable label is a radioactive label.

72. The method of claim 71, wherein the radioactive label is selected from the group consisting of tritium, carbon-14, and iodine-125.

73. The method of claim 66, wherein the detectable label is a chromophoric label.

1 74. The method of claim 66, wherein the detectable label is a chemiluminescent label.

1 75. A method of selecting a prostate specific antigen activatable prodrug wherein the  
2 prodrug is substantially specific for target tissue comprising prostate specific antigen-  
3 producing cells, the method comprising:

- 4 a) linking a peptide of claim 1 to a therapeutic drug to produce a peptide-drug  
5 composition;  
6 b) contacting the composition with cells of the target tissue;  
7 c) contacting the composition with cells of a non-target tissue; and  
8 selecting complexes that are substantially toxic towards target tissue cells, but  
9 which are not substantially toxic towards non-target tissue cells.

76. A method of determining the activity of prostate specific antigen (PSA) in a  
sample containing PSA, the method comprising:

- a) contacting the sample with a composition comprising a detectably labeled  
peptide of claim 1 for a period of time sufficient to allow cleavage of the peptide;  
b) detecting the detectable label to yield a detection level;  
c) comparing the detection level with a detection level obtained from contacting  
the detectably labeled peptide with a standard PSA sample.

77. A method of imaging PSA-producing tissue, the method comprising:

- 2 a) administering a peptide linked to a lipophilic imaging label to a subject having  
3 or suspected of having a PSA producing associated cell-proliferative disorder;  
4 b) allowing a sufficient period of time to pass to allow cleavage of the peptide by  
5 PSA and to allow clearance of uncleaved peptide from the subject to provide a  
6 reliable imaging of the imaging label; and  
7 c) imaging the subject.

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